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THE EMBRYO SAC AND EMBRYO OF CUCUMIS SATIVUS.*

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Before the present investigation was begun practically no detailed morphological work had been reported on the Cucurbitaceae and as there has been much doubt concerning the systematic position and relationship of the plants constituting this family it seemed to offer an interesting and profitable field for research.

Cucumis sativus was taken by the writer for special investigation as a representative of the group but before the results of the work could be published Kirkwood reported (3.) the results of his work on "The Comparative Embryology of the Cucurbitaceae." In this paper he considers seventeen species, but not Cucumis sativus. Longo has worked on the behavior of the pollen tube (1.) and in his more recent paper (2.) he reports an interesting condition of the pollen tube in Cucurbita pepo which is practically the same as occurs in Cucumis sativus.

Material for study was collected during the summer and fall, killed in chromo-acetic acid, passed through successive grades of alcohol and preserved in 70%. Serial sections were cut 10–12 mic. thick, 10 mic. being the usual thickness. The stains used

* Contributions from the Botanical Laboratory of the Ohio State University, XXII.

were Anilin Safranin and Gentian Violet, Heidenhain's Iron-Alum-Haematoxylin, and Delafield's Haematoxylin, the latter perhaps giving the best results. Care had to be taken with it and the Iron-Alum-Haematoxylin as the embryo sacs and embryos stained so deeply that it was difficult to make out the details unless a large part of the stain was removed. The stages just after fertilization were quite difficult to observe as the pollen tube discharges a quantity of material which stains very deeply and obscures the embryo sac structures.

Orientation for sectioning was not difficult as the ovulary when cut crosswise gives longitudinal sections of a number of ovules. For the older stages only a portion of the ovulary could be sectioned on account of its size.

The cross section of the very young ovulary shows the placentae with minute protuberances which represent the incipient ovules (Fig. 1). The carpel has three placentae, and the ovules are delveoped in six rows which are usually double, but this is somewhat irregular. The tip of the ovule remains straight for only a short time after the appearance of the archesporial cell (Fig. 6). The cells along the outer margin begin to divide more rapidly than those of the inner side. This unequal growth causes the ovule to turn, and this process continues until the micropyle is brought close to the funiculus. Before the megasporocyte has divided and before the integuments have grown over the nucellus the ovule has curved half the distance, and the normal anatropous condition is practically attained when the ovule has reached the megaspore stage (Fig. 5). At this time the characteristic beak which develops at the tip of the nucellus is already becoming prominent.

The integuments lengthen greatly forming a long narrow micropyle into which the neck-like process of the flask-shaped nucellus projects, even to the tip of the integuments.

The archesporium is as usual a single hypodermal cell that terminates the axial row of the nucellus. It can easily be distinguished from the surrounding cells by its greater size and deeper color due to the denser protoplasmic contents (Fig. 6).

By a transverse division the archesporial cell gives rise to two cells, the megasporocyte and primary parietal cell (Fig. 7). The latter continues to divide by both periclinal and anticlinal walls thus forming the parietal layer (Fig. 8) which remains persistent and with adjoining cells keeps on dividing to form the long beak of the nucellus (Fig. 22).

The megasporocyte is carried down into the tissue quite a distance by the development of the parietal layer before any division occurs. The division of the megasporocyte is normal, giving rise to four equal megaspores (Fig. 10). The potential megaspores soon begin to dissolve and the lower or functional

megaspore begins to enlarge (Figs. 11-12). The latter acquires a very large and distinct nucleus with a nucleolus of unusual size. Kirkwood reported to have found in Trichosanthes that after the division of the megasporocyte the upper cell did not again divide but immediately disorganized, while the lower cell again divided transversely, the upper cell of which also disorganized. The ultimate result, however, is the same in both Cucumis sativus and Trichosanthes, that is, the lowest of the megaspores always becomes the functional one.

The embryo sac and its associated structures are quite small in comparison with the very large nucellus. The development proceeds in the normal way, by a longitudinal division of the nucleus of the megaspore (Fig. 13). At this stage often the potential megaspores have not completed their dissolution and remains of the third one can be seen just above the sac. The nuclei arrange themselves at either end of the sac in the center of which is a vacuole across which strands of protoplasm may extend. In the four-celled stage the large irregular vacuole in the center is also prominent. By two successive divisions the eight-celled embryo sac is formed (Figs. 14–15). The synergids are distinct and lie above the egg. In the early stage they are somewhat globular in shape and follow the outline of the sac. They lengthen considerably and at the time of fertilization they are quite long, sac-like structures. The egg is large and extends below the synergids, at first merely protruding a little beyond their base, but before fertilization it becomes much elongated and swollen (Fig. 17). The polar nuclei are unequal in size, the lower one being the larger. They conjugate before the entrance of the pollen tube. No case of double fertilization was observed; if it occurs it must take place sometime after the polar nuclei are in contact. The antipodals are small cells which lie side by side, in the lower end of the sac. They take the stain more deeply than does the egg apparatus, and for this reason it is often difficult to make out their outline. They do not enlarge but remain in place and are quite distinct even after considerable endosperm has developed.

The development of the embryo is quite irregular. The first division of the oospore is transverse and the upper cell does not divide further and may be regarded as a rudimentary suspensor (Fig. 18). At this stage the synergids have begun to dissolve. The second division is by a longitudinal wall, the lower cell alone dividing. Later, one of the lower cells divides by a more or less oblique wall forming a four-celled embryo (Fig. 19) which is almost surrounded by endosperm. Above the embryo the remains of the two synergids can still be seen, although almost dissolved at this time.

The endosperm is continuous in the region of the embryo but in the lower end of the sac it forms only a thin layer. The later divisions of the embryo are irregular; an oval mass of cells is formed from the end of which the cotyledons develop. When the embryo is about in the ten-celled stage walls begin to appear in the endosperm (Fig. 20). Kirkwood found in Lagenaria lagenaria and other species somewhat flask-shaped embryos with prominent end cells which correspond closely to those of the same age in Cucumis (Fig. 21).

The endosperm is not abundant but there is a greater amount around the embryo than elsewhere, often the lower portion of the embryo sac is entirely destitute of it. The endosperm stains more deeply along the peripheral margin and around the embryo where the nuclei and starch grains are more abundant. The embryo, however, takes the stain much more prominently than any of the endosperm cells.

The embryo develops a distinct layer of epidermal cells before any cotyledonary protuberances appear (Figs. 23–24). The embryo develops apically two cotyledons and distinctly shows the root tip before there is any sign of the appearance of the plumule (Fig. 25). The mature embryo sac contains only a small amount of scattered endosperm, the main food for the young plant being stored in the large cotyledons. In the mature embryo the plumule is two-lobed showing the incipient first leaf (Fig. 26).

The microsporangia appear to develop in the usual way from a plate of hypodermal cells. The cells of the sporogenous tissue are easily distinguished from the adjacent cells by their large size, and different reaction to stains. The young anther shows in cross section a single row of three microsporocytes in each microsporangium (Fig. 31); but in longitudinal section the plate shows a considerable length (Fig. 32).

The mature pollen grain has a thick wall with a bulging at opposite sides. The tube nucleus and generative nucleus lie to one side of the grain near each other; the latter takes the stain more deeply than the cytoplasm of the rest of the grain, due to its denser structure.

The behavior of the pollen tube in this species is of special interest. It is large and distinct and with Delafield's Haematoxylin stains an amber color while the surrounding cells are a purplish blue; with other stains it is of a deeper color. It enters the micropyle through the opening at the tips of the integuments, pierces the beak of the nucellus and makes its way down to the embryo sac by following a central path of much elongated clear cells which seem to offer little resistance and serve as a definite conducting tissue. The tube sometimes makes its way with

little deviation (Fig. 27) throughout its entire course; but usually there is a peculiar and characteristic bulging (Fig. 28) some distance above the embryo sac. It spreads out in the surrounding tissue, completely breaking down the cell structure. However, before it reaches the sac it again narrows, sometimes to a greater extent than elsewhere along its course. After it has pierced the sac it turns to one side or widens out into a foot-like process.

The most typical tubes have not only a bulging but decided haustoria-like processes (Fig. 29) which extend out into the cell structure of the nucellus and in some cases even break through the inner integument (Fig. 30). The haustorial prolongations appear to act as absorbing and conducting agents for the food material of the embryo. Longo reports to have observed these processes in his study of cucurbita and believes them correlated with the distribution of starch in these parts. He also reports to have found in Cucurbita pepo a conducting tissue which the pollen tube follows from the stigma to the embryo sac.

The points of especial interest and peculiarity observed in the deveolpment of Cucumis are (1) the long micropyle into which extends the long neck of the flask-shaped nucellus, (2) the presence of two well developed integuments, (3) the anatropous ovule with orthotropous embryo, (5) the small size of the embryo sac and associated structures in comparison with the size of the nucellus, (6) the irregular development of the embryo, and (7) the peculiar behavior of the pollen tube.

The work represented in this paper was carried on under the direction of Prof. John H. Schaffner, to whom I wish to express my sincere thanks for valuable assistance and suggestions.

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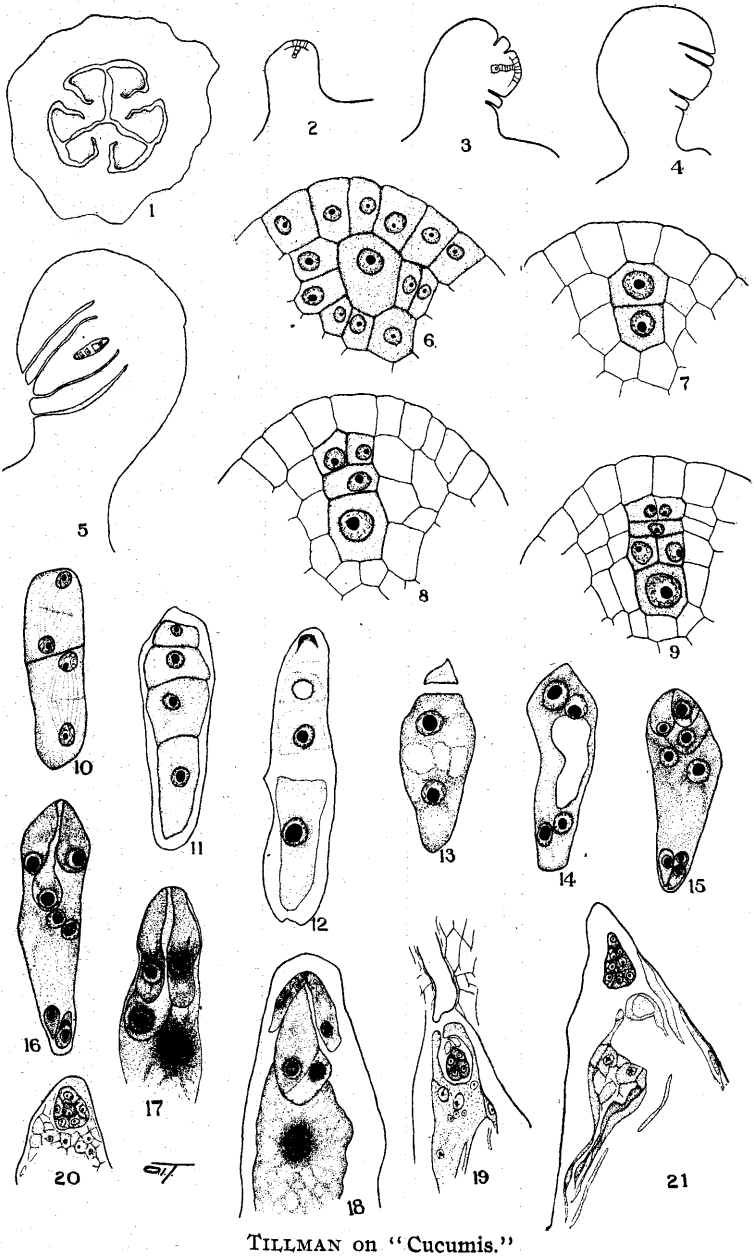
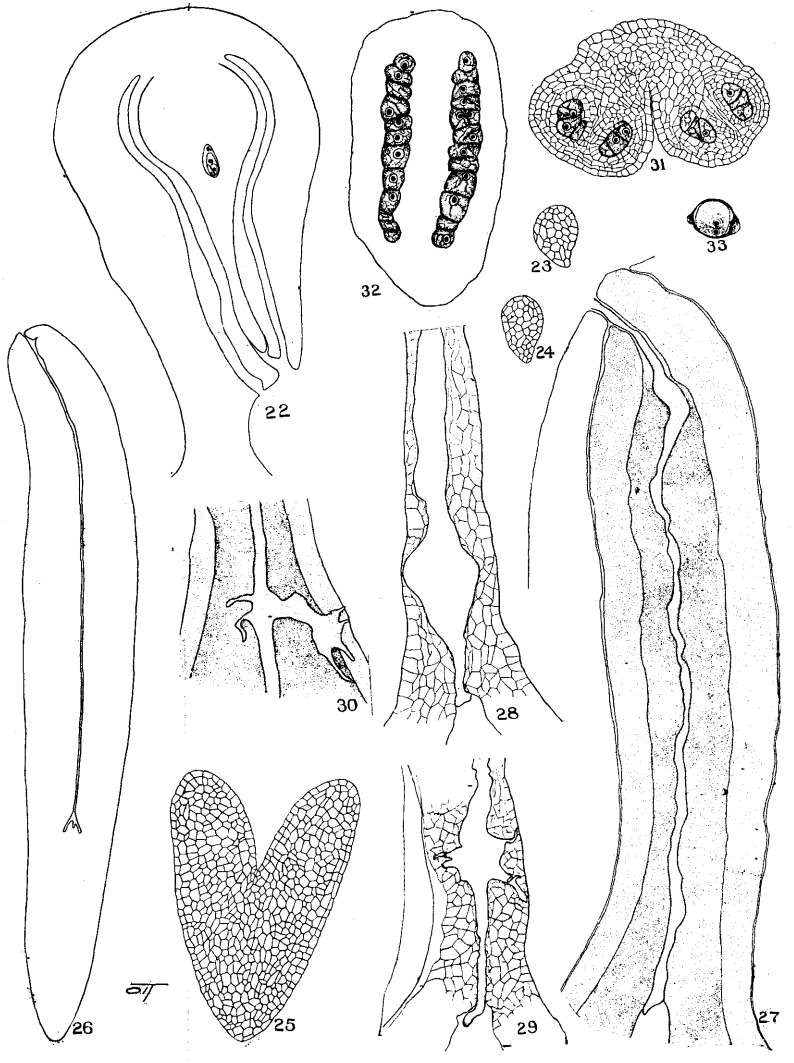
OHIO NATURALIST. *Plate XXIX.*



TILLMAN on "Cucumis."

OHIO NATURALIST. *Plate XXX.*



TILLMAN on "Cucumis."

EXPLANATION OF PLATES XXIX AND XXX.

The drawings were made with the aid of an Abbe camera lucida and various combinations of $\frac{2}{3}$, $\frac{1}{6}$, and $\frac{1}{12}$ oil immersion objectives and No. 2, 1, and $\frac{3}{4}$ oculars.

Fig. 1. Cross section of young ovulary showing incipient ovules.

Figs. 2–5. Series of outlines showing development of integuments and degree of curvature of ovule at different stages.

Fig. 6. Nucellus with archesporial cell.

Fig. 7. Primary parietal cell and megasporocyte.

Fig. 8. Transverse and longitudinal division of parietal layer.

Fig. 9. Further division of parietal layer.

Fig. 10. Second division of megasporocyte producing the four megaspores.

Fig. 11. Potential megaspores beginning to dissolve.

Fig. 12. Enlargement of functional megaspore and further dissolution of three upper megaspores.

Fig. 13. Two-celled embryo sac showing remains of third megaspore.

Fig. 14. Four-celled embryo sac showing large vacuole in center.

Fig. 15. Younger eight-celled embryo sac.

Fig. 16. Older eight-celled embryo sac, showing polar nuclei in contact.

Fig. 17. Upper end of embryo sac just before fertilization, showing large sac-like synergids, and polar nuclei fusing.

Fig. 18. Two-celled embryo, and definitive nucleus.

Fig. 19. Four-celled embryo with endosperm, and remains of two synergids, also pollen tube.

Fig. 20. Young embryo of about ten cells showing irregular division.

Fig. 21. Young embryo and scattered endosperm.

Fig. 22. Outline of eight-celled embryo sac stage, showing micropyle with long beak of nucellus.

Fig. 23. Section of young somewhat spherical embryo.

Fig. 24. Embryo slightly older than that in preceding figure.

Fig. 25. Section of embryo showing cotyledons.

Fig. 26. Outline of mature embryo showing cotyledons, and plumule.

Fig. 27. Entrance of pollen tube into micropyle and course through nucellar beak.

Fig. 28. Entrance of pollen tube into embryo sac, showing peculiar widening near the tip.

Fig. 29. Pollen tube showing enlargement with haustoria-like processes, and bending to one side after entrance into the sac.

Fig. 30. Pollen tube showing the haustoria-like processes extending through inner integument.

Fig. 31. Cross section of anther showing microsporangia and microsporocytes.

Fig. 32. Longitudinal section of anther.

Fig. 33. Mature pollen grain with two nuclei.